Clinical Infectious Diseases

MAJOR ARTICLE

Neonatal Paenibacilliosis: *Paenibacillus* Infection as a Novel Cause of Sepsis in Term Neonates With High Risk of Sequelae in Uganda

Jessica E. Ericson,[1,a] Kathy Burgoine,[2,3,4,a] Elias Kumbakumba,[5,a] Moses Ochora,[5,a] Christine Hehnly,[1,a] Francis Bajunirwe,[5] Joel Bazira,[5] Claudio Fronterre,[6] Cornelia Hagmann,[7] Abhaya V. Kulkarni,[8] M. Senthil Kumar,[9] Joshua Magombe,[10] Edith Mbabazi-Kabachelor,[10] Sarah U. Morton,[11] Mercedeh Movassagh,[9,12] John Mugamba,[10] Ronald Mulondo,[10] Davis Natukwatsa,[10] Brian Nsubuga Kaaya,[10] Peter Olupot-Olupot,[4,13] Justin Onen,[10] Kathryn Sheldon,[1] Jasmine Smith,[1] Paddy Ssentongo,[1] Peter Ssenyonga,[10] Benjamin Warf,[11] Emmanuel Wegoye,[10] Lijun Zhang,[14] Julius Kiwanuka,[5,a,b] Joseph N. Paulson,[15,a] James R. Broach,[1,a] and Steven J. Schiff[12,a]

[1]Department of Pediatrics, Penn State College of Medicine, Hershey, Pennsylvania, USA; [2]Department of Paediatrics and Child Health, Mbale Regional Referral Hospital, Mbale, Uganda; [3]Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom; [4]Mbale Clinical Research Institute, Mbale Regional Referral Hospital, Mbale, Uganda; [5]Department of Pediatrics and Child Health, Mbarara University of Science and Technology, Mbarara, Uganda; [6]Lancaster Medical School, Lancaster University, Lancaster, United Kingdom; [7]Neonatology, University Children's Hospital Zurich, Zurich, Switzerland; [8]Department of Surgery, Hospital for Sick Children, University of Toronto, Toronto, Canada; [9]Harvard T.H. Chan School of Public Health, Dana Farber Cancer Institute, Boston, Massachusetts, USA; [10]CURE Children's Hospital of Uganda, Mbale, Uganda; [11]Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts, USA; [12]Department of Neurosurgery, Yale University School of Medicine, New Haven, Connecticut, USA; [13]Department of Public Health, Busitema University, Busitema, Uganda; [14]Case Western Reserve University School of Medicine, Cleveland, Ohio, USA; and [15]N-Power Medicine, Inc., Redwood City, California, USA

Background. *Paenibacillus thiaminolyticus* may be an underdiagnosed cause of neonatal sepsis.

Methods. We prospectively enrolled a cohort of 800 full-term neonates presenting with a clinical diagnosis of sepsis at 2 Ugandan hospitals. Quantitative polymerase chain reaction specific to *P. thiaminolyticus* and to the *Paenibacillus* genus were performed on the blood and cerebrospinal fluid (CSF) of 631 neonates who had both specimen types available. Neonates with *Paenibacillus* genus or species detected in either specimen type were considered to potentially have paenibacilliosis, (37/631, 6%). We described antenatal, perinatal, and neonatal characteristics, presenting signs, and 12-month developmental outcomes for neonates with paenibacilliosis versus clinical sepsis due to other causes.

Results. Median age at presentation was 3 days (interquartile range 1, 7). Fever (92%), irritability (84%), and clinical signs of seizures (51%) were common. Eleven (30%) had an adverse outcome: 5 (14%) neonates died during the first year of life; 5 of 32 (16%) survivors developed postinfectious hydrocephalus (PIH) and 1 (3%) additional survivor had neurodevelopmental impairment without hydrocephalus.

Conclusions. *Paenibacillus species* was identified in 6% of neonates with signs of sepsis who presented to 2 Ugandan referral hospitals; 70% were *P. thiaminolyticus*. Improved diagnostics for neonatal sepsis are urgently needed. Optimal antibiotic treatment for this infection is unknown but ampicillin and vancomycin will be ineffective in many cases. These results highlight the need to consider local pathogen prevalence and the possibility of unusual pathogens when determining antibiotic choice for neonatal sepsis.

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[a]J. E. E., K. B., E. K., M. O., C. H., J. K., J. N. P., J. R. B., and S. J. S. contributed equally.

[b]Deceased.

Correspondence: J. E. Ericson, Department of Pediatrics, Penn State College of Medicine, 90 Hope Drive, A480, P.O. Box 855, Hershey, PA 17033 (jericson@pennstatehealth.psu.edu).

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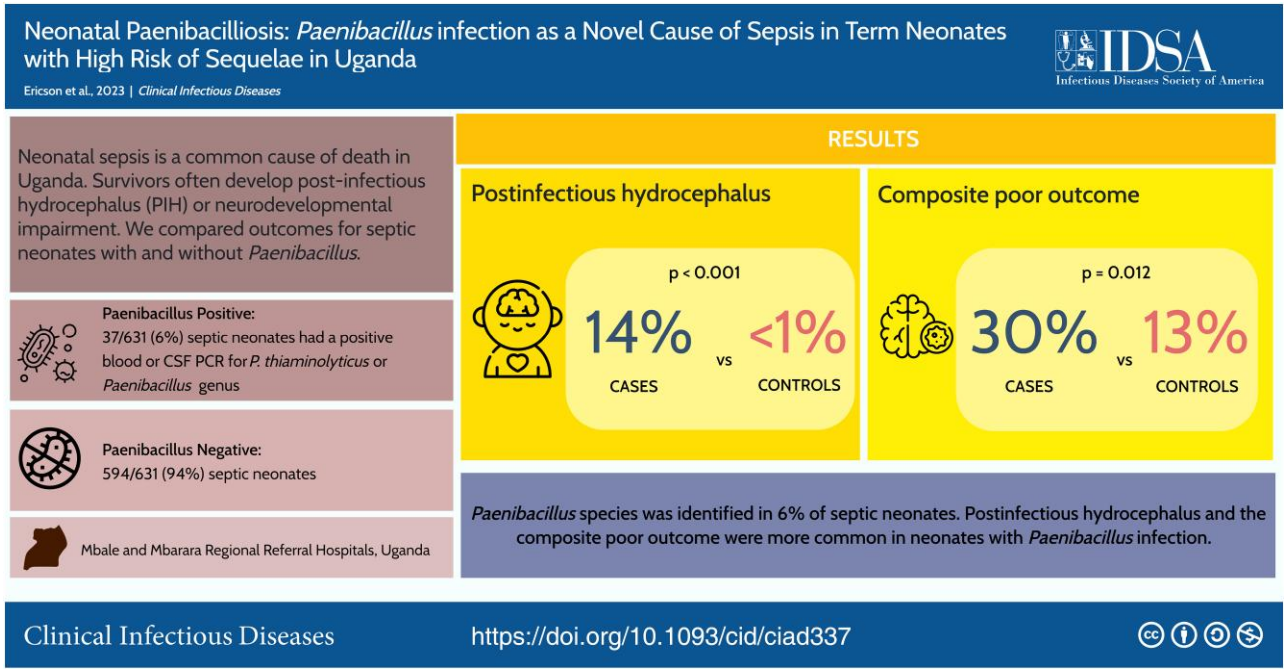
Graphical Abstract



This graphical abstract is also available at Tidbit: https://tidbitapp.io/tidbits/neonatal-paenibacilliosis-paenibacillus-infection-as-a-novel-cause-of-sepsis-in-term-neonates-with-high-risk-of-sequelae-in-uganda

Keywords. meningitis; hydrocephalus; mortality; ampicillin; empirical antibiotic.

Neonatal sepsis (NS) is a leading cause of childhood death worldwide [1]. Affected infants are disproportionately from low-resource settings [1]. Survivors of neonatal sepsis and meningitis have increased risk of neurodevelopmental impairment, hydrocephalus and cerebral palsy [2]. Effective antibiotic therapy relies on identification of the causative pathogen or, in the absence of this information, empirical therapy sufficiently broad to provide effective coverage for the most likely pathogens [2]. In low-resource settings, cultures are often unavailable or uninformative [3]. Culture-independent methods to identify causative pathogens have recently become possible due to advances in molecular diagnostics.

Current international guidelines recommend the combination of ampicillin and gentamicin as first-line empirical antibiotic therapy for neonates with sepsis [4]. In regions where antibiotic resistance is common, these antibiotics are often not the ideal treatment [5]. Without local culture and antibiotic susceptibility testing, the risk of antibiotic resistance is unknown [6, 7].

In previous work, using targeted metagenomics, we found 41% of infants <90 days of age with postinfectious hydrocephalus (PIH), a common sequela of NS in Uganda, had a *Paenibacillus* spp. infection [6]. Here we ask if neonates with *Paenibacillus* detected are more likely to have the composite outcome of PIH, death, or neurodevelopmental impairment than those without *Paenibacillus* detected and characterize the clinical syndrome of neonatal paenibacilliosis.

METHODS

Study Population

Ugandan neonates (≤28 days of age) previously enrolled in a parent study were evaluated for inclusion in this subanalysis focused on *Paenibacillus* infection. The parent study enrolled 800 neonates presenting with clinical signs of sepsis (fever, poor feeding and lethargy; hypothermia, poor feeding, and lethargy; clinical seizures and/or bulging fontanelle, poor feeding, and fever). The study sites were Mbale Regional Referral Hospital in Eastern Uganda and Mbarara Regional Referral Hospital in Western Uganda. Neonates born at a gestational age <37 weeks or <2000 grams birthweight, and those who had been diagnosed with birth asphyxia were excluded. Blood and cerebrospinal fluid (CSF) were collected from each neonate using aseptic technique. Infants were eligible for this subanalysis if they had sufficient blood and CSF available to undergo *Paenibacillus* quantitative polymerase chain reaction (qPCR) testing.

Neonates with PIH were referred to CURE Children's Hospital of Uganda, a neurosurgical specialty hospital located

in Mbale for further management. CSF was collected as part of the neurosurgical evaluation and processed as previously described for culture, genomic sequencing, and qPCR [8].

Laboratory Analysis

An aliquot of each specimen type was collected into DNA/RNA preservative (DNA/RNA Shield, Zymo Corporation) and frozen at −80° C. Additional aliquots of blood and CSF were processed in the local clinical laboratory for standard-of-care clinical tests, and residual aliquots were frozen without preservative at −80° C. Once the entire cohort had been enrolled, the frozen samples were transferred to Penn State University for processing. Both CSF and blood were available for 631/800 (79%) of the neonates enrolled. For these 631, we performed qPCR on 2 μL of processed blood and CSF using primers specific to both the *Paenibacillus* genus and *P. thiaminolyticus*. Neonates with detection of *Paenibacillus* using either test were considered to have possible paenibacilliosis.

Patient and clinical data were prospectively abstracted from each infant's medical record. The presence of seizure-like movements was considered to represent seizures; no electroencephalograms were performed. Fever was defined as any temperature ≥38 C.

Infants underwent developmental assessments at 2 months, 6 months, and 12 months of age using the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III) [9]. Population-normalized BSID-III scores range from 1 to 19 with a population mean of 10 and a standard deviation (SD) of 3. Neurodevelopmental impairment (NDI) was defined as a BSID-III score < −2SD from the mean on any of the subscales.

We compared the demographic characteristics, presenting clinical signs, antibiotic treatment, and clinical course for patients with neonatal paenibacilliosis to septic neonates without evidence of *Paenibacillus* infection using Fisher exact test for proportions and Wilcoxon rank-sum test for continuous variables. Similarly, we compared the proportion of patients with and without paenibacilliosis who experienced inpatient death, infant death (death between birth and 12 months of age), PIH, NDI, and the composite of infant death, PIH, or NDI during the first 12 months of age using Fisher exact test. For neonates with *Paenibacillus*, we used the maximum of genus- or species-level concentration to compare the median concentration detected for neonates with and without hydrocephalus, infant death, and the composite poor outcome. Finally, for infants with paenibacilliosis, we compared the proportion of patients with fever, irritability, clinical seizure, tachypnea, respiratory distress, umbilical cord discharge, hypertonia, tachycardia, bulging fontanelle, and stiff neck between the 2 study sites. Missingness was uncommon, and no adjustment or imputation was undertaken.

Sixty-one infants with PIH had their CSF processed in the same manner, and 3 CSF samples ultimately grew *P. thiaminolyticus*. Susceptibility testing and interpretations were performed by E-test method using Clinical and Laboratory Standards Institute guidelines [8].

This study was approved by the Institutional Review Boards at The Pennsylvania State University, Yale University, CURE Children's Hospital of Uganda, Mbarara University of Science and Technology, and with oversight of the Ugandan National Council on Science and Technology. Written informed consent was obtained from each patient's mother prior to enrollment. All data produced in the current study are available upon reasonable request to the authors and will be made publicly available once the parent neonatal sepsis study is published.

RESULTS

Clinical Epidemiology of Paenibacillosis

Six percent (37/631) of neonates with clinical sepsis had polymerase chain reaction (PCR) evidence of *Paenibacillus* infection. CSF PCR was positive in most cases—35/631 (6%) patients; 1/35 (3%) of these also had a positive blood PCR. Two additional patients had a positive blood PCR but negative CSF PCR (Supplementary Table 1). Most patients (70%) with a PCR positive for *Paenibacillus* genus also had a species-level PCR positive for *P. thiaminolyticus* (Supplementary Figure 1). Quantitative PCR counts varied from 61 copies/mL to 926 764 copies/mL.

Postnatal age at presentation was similar for neonates with and without paenibacilliosis (Table 1). Most neonates with paenibacilliosis were born vaginally (73%) in a healthcare facility (78%). However, when compared to infants without paenibacilliosis, infants with paenibacilliosis detected were significantly more likely to be born at home, 6% versus 22%, respectively, $P < .01$ and to have received non-recommended cord stump care, 19% versus 38%, respectively, $P = .01$.

Neonates with paenibacilliosis frequently had fever (92%) and irritability (84%) (Figure 1). Clinical seizures were present in half (51%) of neonates. Electroencephalograms are not available at these hospitals so subclinical seizures were not identified and some seizure-like movements may not have been true seizures. A bulging fontanelle was present in 22% overall and in 6/16 (38%) infants at Mbale but in only 2/21 (10%) at Mbarara, $P = .06$. All other presenting signs occurred in a similar proportion of neonates at each of the 2 sites.

Laboratory Studies

Aerobic blood cultures grew an organism in 5/37 (14%) of neonates (Supplementary Table 2). Two grew *Staphylococcus aureus*, and 1 each grew a *Klebsiella* species, a *Bacillus* species, and *Streptococcus agalactiae*. One of the neonates with *S. aureus*

Table 1. Demographics of Neonates With Clinical Sepsis due With and Without *Paenibacillus* species Detected by qPCR

	Paenibacillus PCR Positive *N* = 37 (6%)	*Paenibacillus* PCR Negative N = 594 (94%)	*P*
Median age at sepsis presentation, days (25th, 75th percentiles)	3 (1, 7)	2 (1, 4)	.129
<3 d	22 (61)	433 (73)	
3–7 d	6 (17)	55 (9)	
8–14 d	7 (19)	46 (8)	
>14 d	1 (3)	59 (10)	
Median gestational age at birth, wks (25th, 75th percentiles)	39 (38, 40)	40 (39, 41)	.266
Median birth weight, g (25th, 75th percentiles)	3200 (3000, 3500)	3200 (2900, 3500)	.505
Sex			.865
Female	20 (54)	335 (56)	
Male	17 (46)	259 (44)	
Maternal HIV status			.209
Positive	4 (11)	29 (5)	
Negative	31 (84)	539 (91)	
Unknown	2 (5)	27 (5)	
Median maternal age, y (25th, 75th percentiles)	26 (23, 30)	24 (21, 29)	.443
Median maternal parity (25th, 75th percentiles)	2 (1, 4)	2 (1, 4)	.402
Maternal fever during pregnancy	19 (51)	333 (51)	.608
Maternal fever during labor	8 (22)	164 (28)	.487
Delivery location			.003[a]
Healthcare	29 (78)	555 (94)	
Hospital	21 (57)	422 (71)	
Health center	7 (19)	113 (19)	
Clinic	1 (3)	20 (3)	
Home	8 (22)	36 (6)	
Delivery mode			.166
Vaginal	27 (73)	361 (61)	
C-section	10 (27)	230 (39)	
Rupture of membranes >18 h	8 (24)	101 (19)	.495
Umbilical cord care			.011[b]
None	23 (62)	475 (81)	
Any substance	14 (38)	113 (19)	
Saliva	6 (16)	41 (7)	
Cosmetic	6 (16)	35 (6)	
Plant material	2 (5)	28 (5)	
Other	0 (0)	9 (10)	
Feeding method			.123
Exclusively breast fed	34 (92)	561 (94)	
Breast milk and water	1 (3)	1 (<1)	
Breast milk and replacement feeding	1 (3)	20 (3)	
Replacement feeding	1 (3)	4 (1)	
Unknown	0 (0)	8 (1)	
Location			.085
Mbarara	21 (57)	244 (41)	
Mbale	16 (43)	350 (59)	

Abbreviations: HIV, human immunodeficiency virus; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction.

[a]Fisher exact test comparing the proportion of patients with and without *Paenibacillus* detected who were born at home versus in a healthcare facility (clinic, health center or hospital).

[b]Fisher exact test comparing the proportion of patients with and without *Paenibacillus* detected who had any versus no substances applied to the umbilical cord stump.

died shortly after hospital admission. None of the neonates had a blood test positive for malaria or cytomegalovirus. No CSF culture grew bacteria in the local laboratory.

Treatment

Empirical therapy varied across the cohort. Most neonates with paenibacilliosis (26/37, 70%) were initially started on intravenous ampicillin plus gentamicin. The empirical antibiotic therapy was escalated to a broader antibiotic regimen for 9/37 (24%) neonates. Antibiotic escalation occurred after a median of 3 days (IQR: 3, 3). Most commonly, ampicillin was changed to a cephalosporin (5/9, 56%).

Outcomes

The composite poor outcome of infant death, PIH, or NDI was more common in neonates with paenibacilliosis than those without, 11/37 (30%) versus 79/594 (13%), $P = .012$. Among neonates with paenibacilliosis, there was no difference in the frequency of the composite poor outcome between the two sites, 7/16 (44%) at Mbale and 4/21 (19%) at Mbarara, $P = .151$ (Figure 2). Infant death following paenibacilliosis occurred in 5/37 (14%). Three patients progressed rapidly to death prior to hospital discharge. Another infant remained critically ill during the hospital stay and was discharged home against medical advice; this infant died shortly after discharge. A fifth patient was treated with 5 days of ampicillin and gentamicin; he was discharged home from the hospital in good condition but developed a second febrile illness and died at 2 months of age.

PIH was also more common among neonates with paenibacilliosis than those neonates without paenibacilliosis: 5/37 (14%) versus 3/594 (<1%), $P < .001$. Additionally, PIH occurred following paenibacilliosis more frequently at Mbale, 5/16 (31%), than at Mbarara, 0/21 (0%), $P = .010$.

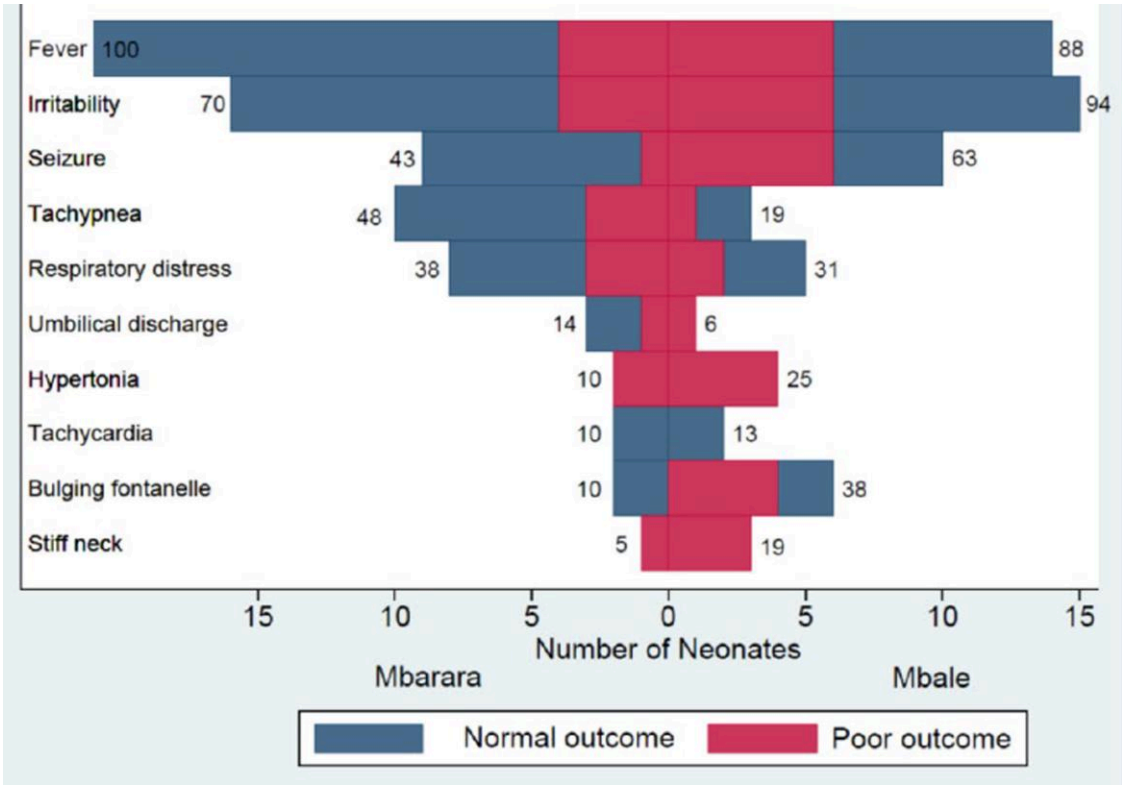


Figure 1. Presenting signs and outcomes for neonates with *Paenibacillus* species detected by qPCR during clinical sepsis. Number of neonates presenting to each site with each sign of infection. Numbers at the end of each bar indicate the proportion of neonates at each site who had the corresponding sign of infection. Neonates with the composite poor outcome of death, postinfectious hydrocephalus or moderate/severe neurodevelopmental impairment are indicated in red. Abbreviation: qPCR, quantitative polymerase chain reaction.

Four of the 5 (80%) neonates with *Paenibacillus*-associated PIH had an elevated CSF protein concentration but all 5/5 (100%) had a CSF white blood cell (WBC) count < 100 $\times 10^6$ cells/L at presentation (Table 2). Two required placement of a ventricular peritoneal shunt, 2 were managed conservatively without surgery, and 1 was referred for neurosurgical evaluation but was lost to follow-up. Three of the 4 patients with paenibacilliosis-associated PIH who remained in care had moderate/severe neurodevelopmental impairment as assessed at 6 or 12 months of age (Supplementary Table 3). One additional neonate (4%) who survived without hydrocephalus had NDI at the last developmental assessment.

Two of the neonates with *Paenibacillus* infection presented to CURE Children's Hospital for surgical management of PIH and had CSF collected a second time [10]. Both patients still had a qPCR positive for *Paenibacillus*. These and an additional 207 infants with PIH seen only at CURE had CSF collected. Three CSF samples ultimately grew *P. thiaminolyticus* [8]. All 3 isolates were susceptible to ceftriaxone, trimethoprim-sulfamethoxazole, tetracycline, and meropenem; none were susceptible to vancomycin, and 2/3 (67%) were resistant to ampicillin (Table 3).

Neonates who died had a similar median (25th, 75th percentiles) log concentration of *Paenibacillus* detected as those who survived: 7.4 (6.0, 10.2) versus 11.0 (7.5, 12,2), respectively, $P = .339$, but those with hydrocephalus and those with the composite poor outcome had more *Paenibacillus* detected than those without: 7.38 (5.64, 10.0) versus 12.4 (10.9, 13.7), $P = .021$, and 7.33 (5.64, 7.95) versus 11.4 (10.0, 12.6), $P = .007$ (Supplementary Figure 2).

DISCUSSION

We describe the first cohort of neonates with sepsis due to *Paenibacillus* species; 70% were due to *P. thiaminolyticus*. Signs of meningitis such as irritability, clinical seizures, and bulging fontanelle were common at presentation. Eleven percent of neonates died during their original in-patient admission. Poor outcomes were common among survivors and occurred more frequently among neonates with *Paenibacillus* than those with clinical sepsis due to other causes, $P = .012$.

P. thiaminolyticus has rarely been reported as a cause of human disease. At the time of discovery from human feces in 1951, it was designated *Bacillus thiaminolyticus* [11]. The first human infection was reported in 2008 when an 80-year-old

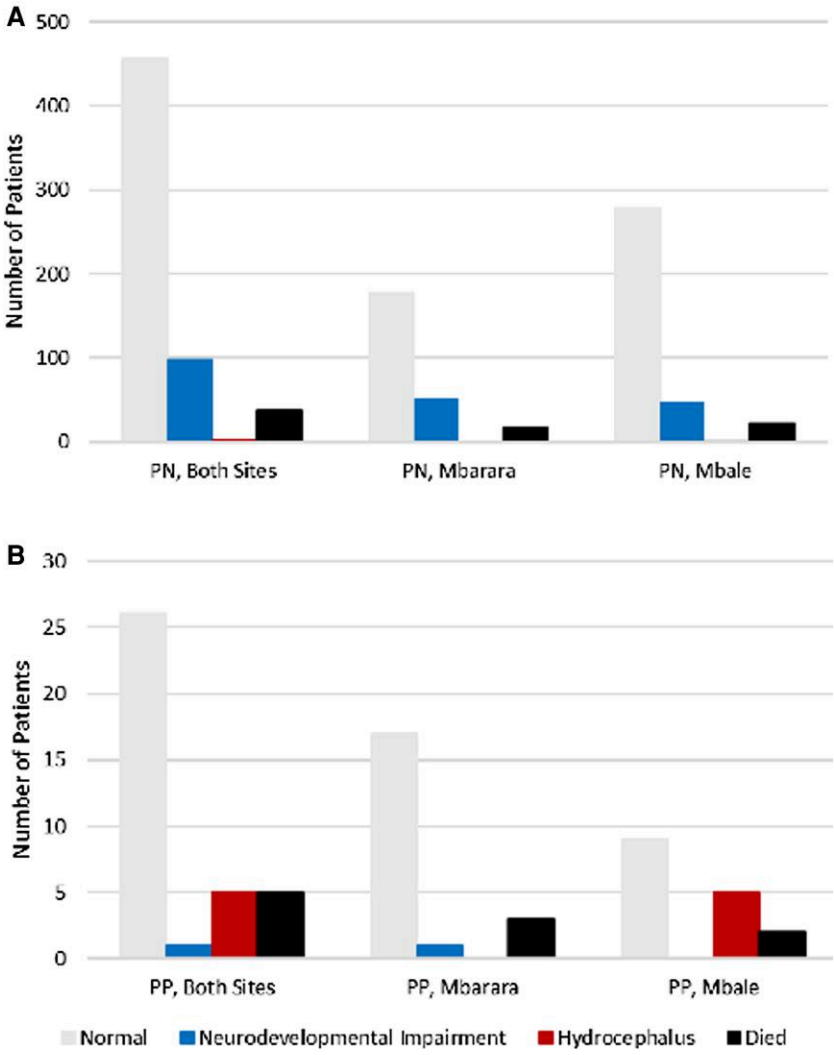


Figure 2. Outcomes for neonates with clinical sepsis with *Paenibacillus* negative (PN) and *Paenibacillus* positive (PP) qPCR results for *Paenibacillus thiaminolyticus* detection in the CSF at both sites and at Mbarara and Mbale. Abbreviations: CSF, cerebrospinal fluid; qPCR, quantitative polymerase chain reaction.

American man undergoing hemodialysis developed *P. thiaminolyticus* bacteremia [12]. He received 4 weeks of vancomycin and improved. A 33-year-old Swiss woman experienced a surgical wound infection due to *P. thiaminolyticus* 7 days following an abdominoplasty [13]. She was treated with 2 weeks of unspecified intravenous antibiotics followed by 2 weeks of amoxicillin-clavulanate and completely recovered. *P. thiaminolyticus* was recovered on blood culture from a 25-day old American neonate presenting with cardiorespiratory arrest following 1 day of poor feeding and increased sleep [14]. Unfortunately, the neonate succumbed to her infection 4 days later. Post-mortem examination revealed a soft brain with several areas of infarction but without clear signs of meningitis. A 16-day old American neonate presented with fever, poor feeding, apnea, and seizures and grew *P. thiaminolyticus* in his CSF [15]. He had severe ventriculitis complicated by thrombosis and required endoscopic third ventriculostomy for management of PIH. He was treated with 6 weeks of meropenem and had residual neurologic sequelae. A 37-day old American infant who had been born at 33 weeks gestation presented to the emergency department with apnea, hypothermia and unresponsiveness. Blood and CSF cultures grew P. thiaminolyticus. He was treated with 21 days of ampicillin but his course was complicated by extensive brain destruction and ischemic stroke. He subsequently developed PIH and had a ventriculoperitoneal shunt placed. He subsequently died at 11 months of age [16]. Finally, in a cohort of 209 Ugandan infants with PIH, we found that Paenibacillus was the most common bacterial genera identified, 91 of 209 (44%) of patients [10]. Our finding that 37 of 631 (6%) neonates evaluated for sepsis had *Paenibacillus sp.* or *P. thiaminolyticus* detected using molecular methods was unexpected and suggests that *P. thiaminolyticus* may be an underdiagnosed cause of neonatal sepsis, meningitis, and PIH in Uganda.

Table 2. Description of Neonates Who Developed Postinfectious Hydrocephalus Following Neonatal Sepsis With *P. thiaminolyticus* Detected by qPCR

	1	2	3	4	5
Age at presentation	9 d	2 d	12 d	6 d	6 d
Sex	Female	Male	Male	Male	Male
Gestational age at birth, wks	38	43	Unknown	38	Unknown
Birth location	Home	Home	Home	Home	Home
Cord care	Cosmetic powder	Cosmetic powder	Vaseline	None	None
Presenting signs	Fever, poor feeding, irritability, bulging fontanelle, lethargy, seizure, umbilical discharge	Fever, poor feeding, irritability, bulging fontanelle, lethargy, seizure, hypertonia	Fever, poor feeding, irritability, bulging fontanelle, lethargy, respiratory distress, hypotonia	Fever, poor feeding, irritability, lethargy, vomiting, seizure, hypotonia	Fever, poor feeding, irritability, lethargy, seizure, stiff neck, hypertonia
CSF cell count, 10^6 cells/L	≤5	75	80	≤5	≤5
CSF protein, g/L	3.4	1.8	0.5	1	2.3
Blood culture result	Negative	Negative	Negative	Negative	Negative
Admission heart rate, beats/minute	125	148	138	136	131
Admission respiratory rate, breaths/minute	36	51	67	59	47
Initial antibiotic treatment	3 d ampicillin/gentamicin,	2 d ampicillin/gentamicin	2 d ampicillin/gentamicin	7 d ceftriaxone/ gentamicin	9 d ampicillin/ gentamicin
Additional antibiotic treatment	11 d ceftriaxone/ gentamicin	7 d ceftriaxone/gentamicin, 14 d ceftriaxone/amikacin 14 d enteral amoxicillin/ ciprofloxacin/metronidazole	4 d cefotaxime/gentamicin, 14 d ceftriaxone/amikacin	…	…
Hydrocephalus treatment	None	Shunted	Shunted	None	None
Paenibacillus PCR result at surgery	Not done	Positive	Positive	Not done	Not done
6-month outcome	Moderate cognitive and motor impairment	Not seen	Moderate cognitive and motor impairment	Normal	Not seen
12-month outcome	Not seen	Moderate cognitive and motor impairment	Not seen	Normal	Not seen

Abbreviations: CSF, cerebrospinal fluid; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction.

Table 3. Antibiotic Susceptibility Testing Results for *Paenibacillus Thiaminolyticus* Isolates Collected From the Cerebrospinal Fluid of Patients With Postinfectious Hydrocephalus as a Sequela of Neonatal Sepsis[a]

Antibiotic	Susceptible N = 3 (%)
Ampicillin	1 (33)
Ceftriaxone	3 (100)
Ciprofloxacin	3 (100)
Clindamycin	0 (0)
Gentamicin	3 (100)
Penicillin	2 (67)
Meropenem	3 (100)
Tetracycline	3 (100)
Trimethoprim-sulfamethoxazole	3 (100)
Vancomycin	0 (0)

[a]Modified from results reported previously [8].

Studies seeking to identify causative organisms for neonatal sepsis may fail to identify *P. thiaminolyticus* for several reasons. First, this organism may have ecological niches that are not universally distributed. We failed to find any evidence of *P. thiaminolyticus* presence in the vaginal microbiome of 99 women residing in Mbale or Mbarara, Uganda, at the time of delivery, suggesting that neonates may become colonized with the organism through environmental, rather than maternal sources [17]. It has been identified in fish from Lake Michigan in the United States [18] and from the soil in India [19]. Other species of *Paenibacillus* have been identified in the soil globally [20, 21] and as a member of the human gut microbiome [22]. Some species are known to infect honeybees and, rarely, humans [23]. Neonates cared for in industrialized or high-resource settings may have limited contact with environmental reservoirs of *Paenibacillus*. The diagnosis of *Paenibacillus* infections is also complicated by variable gram staining. Although the organism is phylogenetically a gram-positive, it sometimes stains as a gram-negative.

Even common neonatal pathogens can be missed when the blood volume used to inoculate the blood culture bottles is <1 mL [24]. Low sample volume will similarly limit our ability to detect *Paenibacillus*. We were able to grow *P. thiaminolyticus*

from the CSF of 3 neonates with PIH by inoculating the CSF into anaerobic lytic blood culture bottles [8]. Anaerobic blood culture bottles are not used as a routine part of the evaluation of neonatal sepsis in most clinical settings but have been shown to increase the diagnostic yield of blood cultures for neonates with bacteremia [25]. The addition of an anaerobic blood culture bottle to the CSF evaluation of neonates with sepsis may improve the diagnosis of infections due to *Paenibacillus* and facultative anaerobes implicated in neonatal sepsis.

The diagnosis of *Paenibacillus* infection is important for the care of neonates with sepsis due to the high risk of mortality and morbidity. In contrast to infections due to *Streptococcus agalactiae* (0%–4% [26]), paenibacilliosis resulted in PIH in 12% of cases. Meningitis due to *Escherichia coli* causes PIH in 18%–22% [26]. *Paenibacillus* conferred a similar risk of PIH as *E. coli* but was a more common cause of infection in our cohort (data not shown). Thus, paenibacilliosis may be a leading cause of PIH in Uganda. It is unknown whether optimal treatment of neonatal paenibacilliosis would reduce the incidence of PIH or NDI in the region. We included death from any cause up to 12 months of age in our composite outcome because sequelae of the sepsis episode could increase the risk of death due to malnutrition, seizures, or other causes. Some deaths outside of the neonatal period may have been unrelated to neonatal sepsis and its sequelae.

The World Health Organization recommends using the combination of ampicillin and gentamicin as empirical therapy for neonatal sepsis due to the coverage provided against *S. agalactiae*, *Escherichia coli*, and *Listeria monocytogenes* [4]. Regions where antibiotic resistance is common or additional organisms are typical may need to use a broader spectrum regimen empirically. Our discovery that *P. thiaminolyticus* could be a common cause of culture-negative sepsis in Uganda has important implications for empiric antibiotic selection. Antibiotic susceptibility testing performed on the three isolates we successfully isolated from neonates with PIH demonstrated resistance to ampicillin and vancomycin. We additionally identified the presence of several beta-lactamase genes which could confer resistance to ampicillin [8]. Prior studies have found that resistance to ampicillin, vancomycin, clindamycin, and tetracycline is common in *Paenibacillus* species [13]. Because there are few clinical reports of *P. thiaminolyticus*, it is unknown how often resistance occurs or what antibiotic regimens would maximize the likelihood of a good outcome.

While we await clinical studies to inform antibiotic therapy, the combination of a third-generation cephalosporin and gentamicin would be preferred over ampicillin and gentamicin as an empiric antibiotic regimen for neonatal sepsis in Uganda especially when meningitis is suspected. A prior study of soil *Paenibacillus* species suggests that ~70% of isolates are susceptible to ceftriaxone, an antibiotic that has good penetration into the central nervous system [27]. Antibiotic susceptibility testing should be performed to guide antibiotic management in individual or regional cases of paenibacilliosis.

Vancomycin is commonly used for gram positive central nervous system (CNS) infections and is the drug-of-choice for CNS infections due to *Bacillus species* but should not be used empirically for treatment of *Paenibacillus* infections. It is possible that alternate antibiotic regimens may be optimal for treatment of this novel infection. Because this organism may be an important pathogen in the developing world, any antibiotic consideration would need to achieve therapeutic concentrations in the CNS, be inexpensive, easily administered, and well tolerated with few side effects.

Nutritional interventions may be important adjunctive treatments for *P. thiaminolyticus* infections. This organism produces thiaminase, an uncommon bacterial enzyme which has the potential to reduce thiamine levels [16]. Thiamine supplementation may have a role for certain types of infection, in cases when sepsis is caused by a thiamine-consuming organism or when pre-existing thiamine deficiency exists. Furthermore, thiamine supplementation may improve neurodevelopmental outcomes or reduce the likelihood of other sequelae, such as PIH, following neonatal sepsis even if it does not confer a survival benefit [28]. Studies evaluating the role of thiamine supplementation as adjunctive therapy for neonatal sepsis due to thiaminase-producing *P. thiaminolyticus* are needed.

Future research will need to define the qPCR level that is clinically significant. Very low copy numbers may be due to contamination at the bedside or in the laboratory. Alternatively, low copy numbers could reflect that *Paenibacillus* is a commensal bacterium but is not the cause of disease. In this report, we included all neonates who had any *Paenibacillus* detected. It is possible that some infants included had infections due to other organisms, coinfections with *Paenibacillus*, or even noninfectious reasons for presenting with clinical signs of sepsis.

CONCLUSION

Paenibacillus species was identified in 6% of neonates with signs of sepsis who presented to 2 Ugandan referral hospitals; most were *P. thiaminolyticus*. Improved diagnostics for neonatal sepsis are urgently needed. Optimal antibiotic treatment for this infection is unknown but ampicillin and vancomycin will be ineffective in many cases.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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